



## **Sequencing primers and protocol**

### **28 April 2009**

The WHO Collaborating Centre for influenza at CDC Atlanta, United States of America, has made available the sequencing primers (table attached) and protocol as follows.

1. RNA extraction performed on 100 ul viral material (cell or egg) and eluted in 100 ul volume.
2. Only need to prepare 10-15 ul PCR reactions using 1 ul RNA
3. Our cycling conditions using the Promega Access Quick RT-PCR kit is as follows:
  - Cycle 1: 48 C for 45'
  - Cycle 2: 94 C for 2'
  - Cycle 3: 94 C for 20s
  - Cycle 4: 50 C for 30s
  - Cycle 5: 72 C for 1'
  - Cycle 6: Go to Cycle 3 29X
  - Cycle 7: 72 C for 7'
  - Cycle 8: 4 C hold
4. Product size verified on agarose gel
5. Prepare each amplicon to be sequenced with each of M13-forward and M-13 reverse and perform sequence reactions and clean up according to your instruments protocol.

## Swine Genome Primer Set

	<b>forward</b>	<b>5'</b>	<b>3'</b>	<b>reverse</b>	<b>5'</b>	<b>3'</b>
<b>PB2</b>						
fragment 1	<b>1</b>	tgt aaa acg acg gcc agt agc aaa agc agg tca att		<b>538</b>	cag gaa aca gct atg acc ctt cca tra tta cat cyt gtg	
fragment 2	<b>328</b>	tgt aaa acg acg gcc agt gtr aca tgg tgg aay aga a		<b>816</b>	cag gaa aca gct atg acc gct ttg rtc aay atc rtc att	
fragment 3	<b>487</b>	tgt aaa acg acg gcc agt cct ggt cay gca gac ctc ag		<b>1019</b>	cag gaa aca gct atg acc cca aar ctg aag gay gar ctg at	
fragment 4	<b>713</b>	tgt aaa acg acg gcc agt caa gca gtr trt aca ttg aag t		<b>1289</b>	cag gaa aca gct atg acc cct cta act gct ttt ayc atg caa t	
fragment 5	<b>946</b>	tgt aaa acg acg gcc agt ccr acw gaa gaa caa gct gt		<b>1509</b>	cag gaa aca gct atg acc gga gta ttc atc yac acc cat	
fragment 6	<b>1169</b>	tgt aaa acg acg gcc agt aag caa cca gra gat tgr ttc a		<b>1740</b>	cag gaa aca gct atg acc ctg aga cca ytg aat ttt rac a	
fragment 7	<b>1447</b>	tgt aaa acg acg gcc agt cca agy acm gag atg tca atg aga		<b>2186</b>	cag gaa aca gct atg acc ttr ctc art tca ttg atg ct	
fragment 8	<b>1683</b>	tgt aaa acg acg gcc agt caa tac cta yca rtg gat cat cag aa		<b>2341</b>	cag gaa aca gct atg acc tag tag aaa caa ggt cgt t	
<b>PB1</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>22</b>	tgt aaa acg acg gcc agt agc aaa agc agg tca att		<b>477</b>	cag gaa aca gct atg acc ctr aaw act tct atr gtg tt	
fragment 2	<b>233</b>	tgt aaa acg acg gcc agt caa ctc aac ccr att gat ggr cca ct		<b>843</b>	cag gaa aca gct atg acc gtt caa gct ttt crc awa tg	
fragment 3	<b>389</b>	tgt aaa acg acg gcc agt caa agr gtg gac aaa tra c		<b>1041</b>	cag gaa aca gct atg acc ctg aac cay tca ggy tga ttt	
fragment 4	<b>711</b>	tgt aaa acg acg gcc agt tga aca cra tga cca arg a		<b>1278</b>	cag gaa aca gct atg acc ttg aac atg ccc atc atc aty cca gg	
fragment 5	<b>974</b>	tgt aaa acg acg gcc agt aat caa aay cct mga atg tt		<b>1566</b>	cag gaa aca gct atg acc agc tcc atg ctr aaa ttr gc	
fragment 6	<b>1139</b>	tgt aaa acg acg gcc agt caa ata ccy gca gar atg cta gc		<b>1659</b>	cag gaa aca gct atg acc cca agr tca ttg ttt atc at	
fragment 7	<b>1489</b>	tgt aaa acg acg gcc agt atg agy aaa aag aag ty ta		<b>1954</b>	cag gaa aca gct atg acc tca aty tcy tta tgg gtg ac	
fragment 8	<b>1532</b>	tgt aaa acg acg gcc agt gcy aat tty agc atg gag ct		<b>2321</b>	cag gaa aca gct atg acc agt aga aac aag gca ttt	
<b>PA</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>0</b>	tgt aaa acg acg gcc agt agc aaa agc agg tac tga t		<b>493</b>	cag gaa aca gct atg acc tar tck gcc ttt gtg gcc att tc	
fragment 2	<b>235</b>	tgt aaa acg acg gcc agt cca aat gca ctk tta aar cac aga tt		<b>756</b>	cag gaa aca gct atg acc tga gaa agc ttg ccc tca atg	
fragment 3	<b>361</b>	tgt aaa acg acg gcc agt tat gay tac aar gag aa		<b>989</b>	cag gaa aca gct atg acc ggt tct ttc cat cca aag aat gtt	
fragment 4	<b>702</b>	tgt aaa acg acg gcc agt tgc mtt gar aat ttt agr acc ta		<b>1292</b>	cag gaa aca gct atg acc tcr cak gcc ttg ttg aac tca tt	
fragment 5	<b>894</b>	tgt aaa acg acg gcc agt aaa ttr agc att gar gay cca		<b>1662</b>	cag gaa aca gct atg acc tcw agt cty ggg tca gtg ag	
fragment 6	<b>1204</b>	tgt aaa acg acg gcc agt taa gcg att tra agc aat atg a		<b>2037</b>	cag gaa aca gct atg acc aay ccy tcy aat tgt ggw gat g	
fragment 7	<b>1444</b>	tgt aaa acg acg gcc agt aat gca tcc tgt gca gca atg ga		<b>2057</b>	cag gaa aca gct atg acc ttg tcc cta aga gcc tga aca a	
fragment 8	<b>1787</b>	tgt aaa acg acg gcc agt atg aar tgg gga atg gag atg ag		<b>2233</b>	cag gaa aca gct atg acc agt aga aac aag gta cct ttt	
<b>HA</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>1</b>	tgt aaa acg acg gcc agt ata cga cta gca aaa gca ggg g		<b>461</b>	cag gaa aca gct atg acc tca tga ttg ggc cay ga	
fragment 2	<b>351</b>	tgt aaa acg acg gcc agt acr tgt tac ccw ggr gat ttc a		<b>943</b>	cag gaa aca gct atg acc gaa akg gga grc tgg tgt tta	
fragment 3	<b>379</b>	tgt aaa acg acg gcc agt atg arg arc tra gag agc a		<b>1204</b>	cag gaa aca gct atg acc caa tgg crt tyt gtg tgc tc	
fragment 4	<b>736</b>	tgt aaa acg acg gcc agt agr atg rac tat tac tgg ac		<b>1340</b>	cag gaa aca gct atg acc ttc tkc att rta wgt cca aa	
fragment 5	<b>1124</b>	tgt aaa acg acg gcc agt tgg atg gta ygg tta yca yca		<b>1541</b>	cag gaa aca gct atg acc tca taa gty cca ttt ytg a	
fragment 6	<b>1204</b>	tgt aaa acg acg gcc agt aag atg aay acr car ttc aca g		<b>1778</b>	cag gaa aca gct atg acc gtg tca gta gaa aca agg gtg ttt	
<b>NP</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>1</b>	tgt aaa acg acg gcc agt cag ggt aga taa tca ctc ac		<b>553</b>	cag gaa aca gct atg acc aga gca cat yct ggg atc cat	
fragment 2	<b>296</b>	tgt aaa acg acg gcc agt atg gtr ctc tct gct ttt gat ga		<b>757</b>	cag gaa aca gct atg acc ttt gtg cag ctg ttt gaa att tyc ctt t	
fragment 3	<b>513</b>	tgt aaa acg acg gcc agt tgg cat tch aat ttr aat gat		<b>1042</b>	cag gaa aca gct atg acc ctg rct ctt gtg tgc dgg	
fragment 4	<b>619</b>	tgt aaa acg acg gcc agt gct gca gtc aar gga rt		<b>1177</b>	cag gaa aca gct atg acc aag cra ttt gta cyc ctc tag t	
fragment 5	<b>925</b>	tgt aaa acg acg gcc agt cct gcy tgt ygq taw gga c		<b>1565</b>	cag gaa aca gct atg acc agt aga aac aag ggt att ttt c	
<b>NA</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>0</b>	tgt aaa acg acg gcc agt agc aaa agc agg agt		<b>600</b>	cag gaa aca gct atg acc ctg gac crg aaa ttc c	
fragment 2	<b>318</b>	tgt aaa acg acg gcc agt tac aca aaa gac aay agc		<b>740</b>	cag gaa aca gct atg acc ggr cca tcg gtc att atg	
fragment 3	<b>536</b>	tgt aaa acg acg gcc agt ggt cag caa gcg cat gyc atg a		<b>1063</b>	cag gaa aca gct atg acc cat aty tgt atg aaa acc	
fragment 4	<b>726</b>	tgt aaa acg acg gcc agt aat ggr car gcc tcr tac aa		<b>1346</b>	cag gaa aca gct atg acc gct gct ycc rct agt cca gat	
fragment 5	<b>941</b>	tgt aaa acg acg gcc agt tag gat aca tct gca gtg g		<b>1452</b>	cag gaa aca gct atg acc agt aga aac aag gag	
<b>M</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>0</b>	tgt aaa acg acg gcc agt agc aaa agc agg tag		<b>473</b>	cag gaa aca gct atg acc gca atc tgy tca cak gt	
fragment 2	<b>223</b>	tgt aaa acg acg gcc agt cac cgt gcc cag tga gcg		<b>750</b>	cag gaa aca gct atg acc tca ytt gaa ycg ytg cat	
fragment 3	<b>383</b>	tgt aaa acg acg gcc agt tct gct ggw gca ctt gcc agt tg		<b>1027</b>	cag gaa aca gct atg acc agt agm aac aag gta gt	
<b>NS</b>	<b>forward</b>			<b>reverse</b>		
fragment 1	<b>24</b>	tgt aaa acg acg gcc agt agc aaa agc agg gtg aca aag aca		<b>482</b>	cag gaa aca gct atg acc tcg gtg aaa gcc ctt a	
fragment 2	<b>250</b>	tgt aaa acg acg gcc agt tga ggc wyt taa aat gac ca		<b>890</b>	cag gaa aca gct atg acc agt aga aac aag ggt gtt ttt tat	
fragment 3	<b>418</b>	tgt aaa acg acg gcc agt aaa gcd aay ttc agt gtg		<b>742</b>	cag gaa aca gct atg acc ttc aat hag cca tct ta	

m13 forward      m13 forward  
m13 reverse      m13 reverse